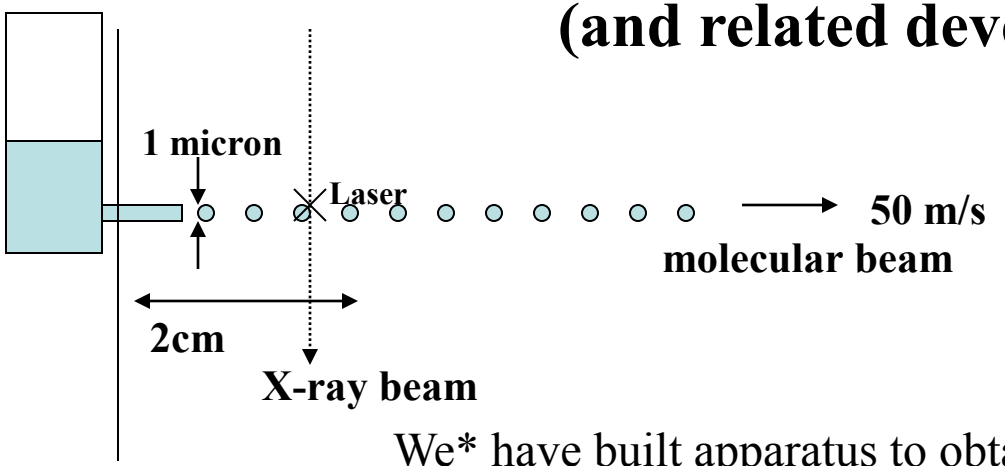
Serial Crystallography
(and related developments)



We* have built apparatus to obtain X-ray diffraction patterns from a beam of ice-jacketted proteins

AIM: To solve protein structures which cannot be crystallized.

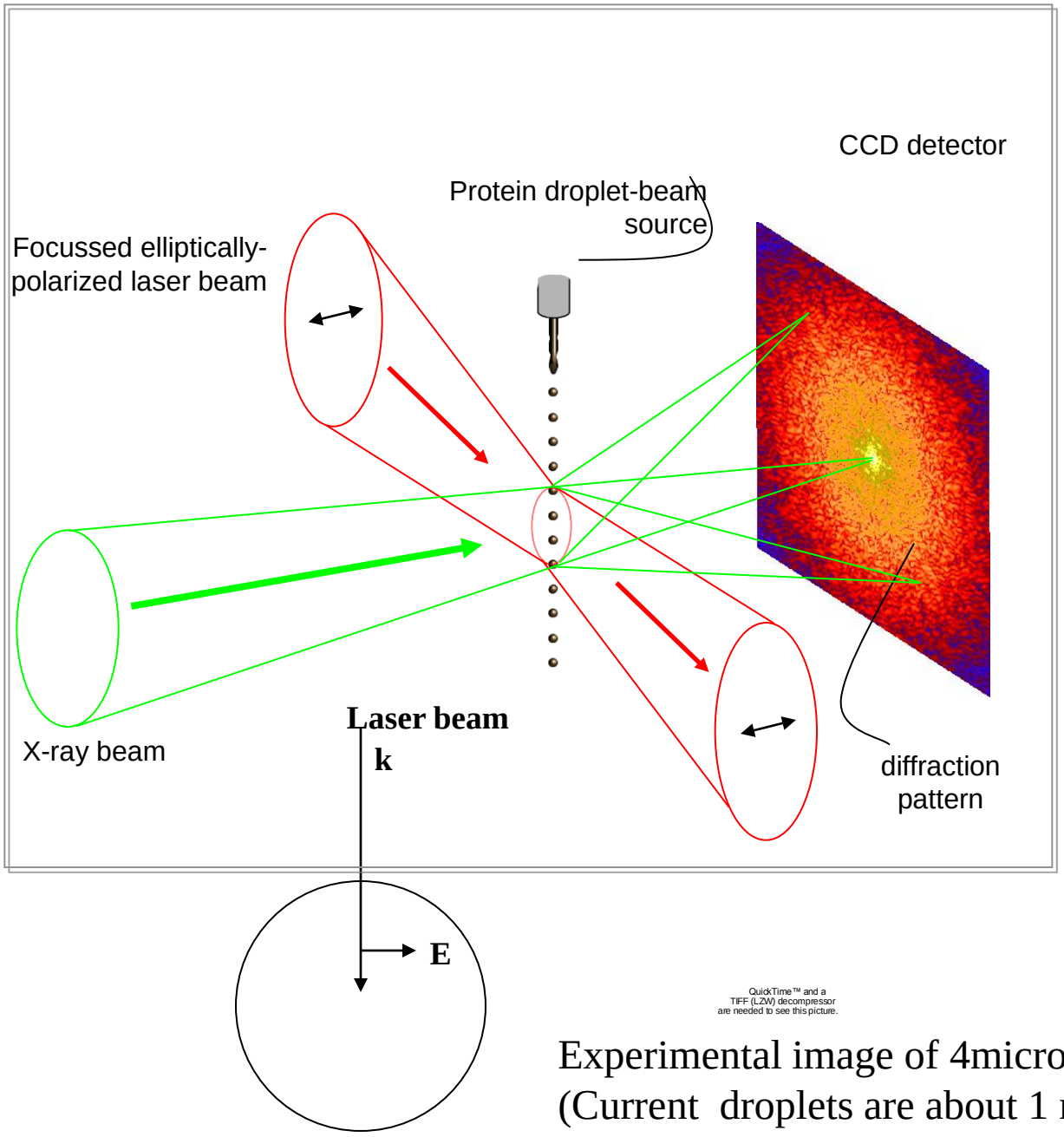
"The most important problem in structural biology"

1. **Serial Crystallography**
2. **Injectors**
3. **Tests with powder, capsid**
4. **Correlation analysis of SAX-PLUS**
5. **Recent results from Flash**

Spence and Doak, P.R.L. 92, 198102 '04. Acta A61, 237. '05. J.Chem Phys 123, 244304 '05

***Spence, Doak, Weierstall, Starodub, DePonte, Schmidt, Fromme,Hembree. ASU NSF IDBR $.**

***Chapman, Spence, Howells, Shapiro, Weierstall, Doak. ALS, ASU XRD CBST $**



Three droplet beam methods

1. Rayleigh
2 Electrospray.
3 Aerojet.

Four alignment methods

1. **Laser**
2. Static field
3. Magnetic field
4. Flow alignment.

Piezo
1MHz

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Experimental image of 4micron droplet beam
(Current droplets are about 1 micron diam)

Serial Xtallography

Consider a single-file stream of identical , aligned molecules traversing an X-ray beam. Assume their spacing exceeds the width of the Xray beam, so there is only one mol in the beam at any instant.

Because the *motion* of one molecule across the collimated X-ray beam has no effect on the diffraction pattern, the pattern is equivalent to that from one immortal, stationary molecule, which never damages*.

Spence and Doak, PRL 2004

*The transit time for one mol travelling at 50m/sec across a ten micron beam is 200ns.

Experimental demonstration of laser-alignment of a molecular beam

***The non-resonant laser induces a dipole moment p for polarizability α.**

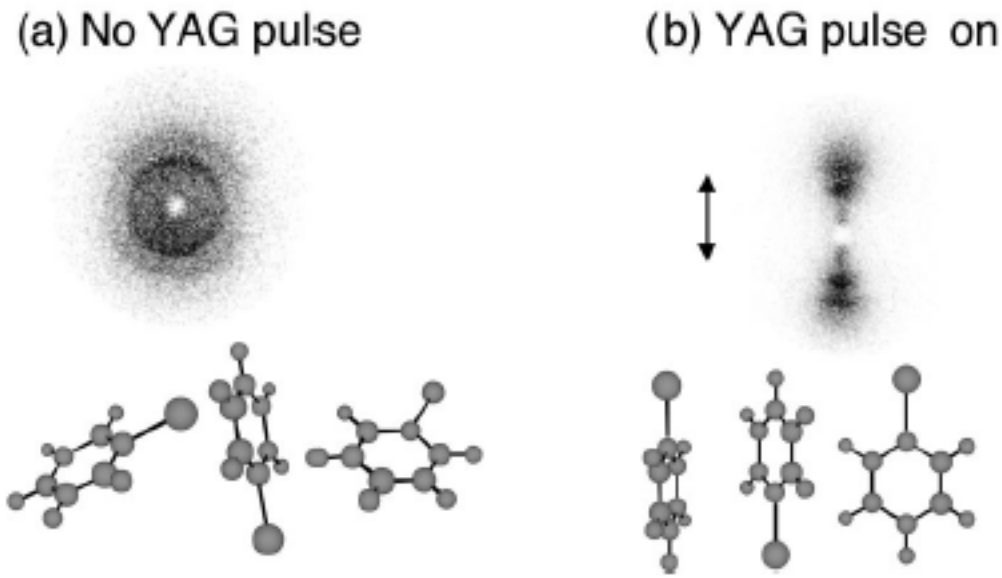
$\mathbf{p_i(t)} = \alpha_{ij}(\theta)\, E_j(t)$

***Because the induced moment is not parallel to the field which caused it, there is a torque τ**

Torque is $\tau = \mathbf{p} \times \mathbf{E}$

When elliptically polarized light is used, all 3 molecular axes are aligned in direction, but not sense (Elser 06).

There are no absorption lines in protein at one micron. At 10^8 watts/cm^2 no damage is expected.



Molecules of Iodobenzene in a gas jet aligned by linearly polarized light. After alignment, mols are ionized and exploded by circularly polarized laser pulse, producing the pattern shown.

Larsen, J. Chem Phys 111, 7774 (1999); Stapelfeldt, Seideman, '03 etc.

Rotational temp 20 K

Electron diffraction from laser-aligned molecules

QuickTime™ and a
decompressor
are needed to see this picture.

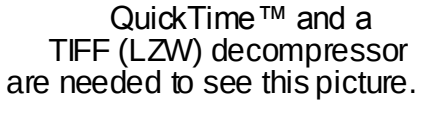
QuickTime™ and a
decompressor
are needed to see this picture.

$C_2F_4I_2$

Reckenthaeler et al. Phys Rev Letts 102, 213001 (2009)

Pump-probe with delay less than 2.6 ps

Charge-density maps can be reconstructed from diffraction patterns from partially-aligned molecules using new iterative phasing methods



GroEl

Exposure times* (Full HIO sim with noise. Scat Fac. for GroEL)

0. ERL (Cornell, planned) 8kV, Flux 3E8

NSLS 2 ?

Resolution	N	Exp time
2nm	1	30 seconds
0.7 (α-helix)	10	204 sec.

1. Current APS capability, 4.5 kV Undulator, Flux 7.7E5 ph/sec/nm^{2}.(0.1% BW)

Resolution	Number of mols. N	Exp Time.
0.7 nm	1	2.5E5 sec
2nm	1	3.8E3
1nm	10	6.1E3 =1.6 hrs

2. Summer 07 ALS upgrade. 1.5 kV, Flux 1E4 ph/sec/nm^{2}.

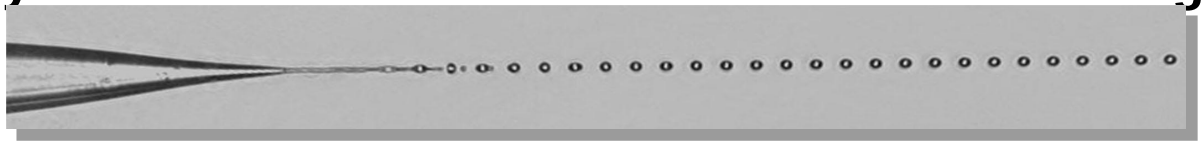
Resolution	Number of mols.	Exp time.
2 nm	100	324 sec
1.5 nm	5	2E4

Divide this exposure time by 1000 if ALS 9.0.1 optimized for our experiment.

*Scaling: $T = s^2 k / (N*I_o\ d^4\ \lambda^2)$ with k = 9.8E6 (nm, sec) . Oversampling ratio s -sqrt (2) above. For constant T, d goes as $N^{-1/4}$. Analytic result (Rez, Howells) for S/N=5 gives 100 times shorter

Injectors for hydrated particles.

Over past 3 years we have built and evaluated the following types at ASU.



Electrospray - Sheds water by coulomb explosion, Fenn.
Proteins may be charged and so unfold
Very low particle density (mist)
Cannot be synchronized. Is used in current TOF of proteins.

Rayleigh jet - High particle density (single file) cf Ink Jets
Don't shed water.
Small nozzles (<10 microns) clog. Droplets twice nozzle diam
Can be synchronized (driven by piezo)
Not charged.

Nebulizer - Low particle density (mist)
Not charged
Can be synchronized (piezo)
Don't shed water, but smaller droplets.

AIM: To collect data from proteins which cannot be crystallized, especially membrane Proteins. (P.Fromme)

Aerojet - Water shedding difficult so far
Not charged. Monodispered.
High density (single file)
Can be **synchronized**
Does not clog. Mixing experiments possible (protein folding)

We developed the capability for ns imaging of droplet beam

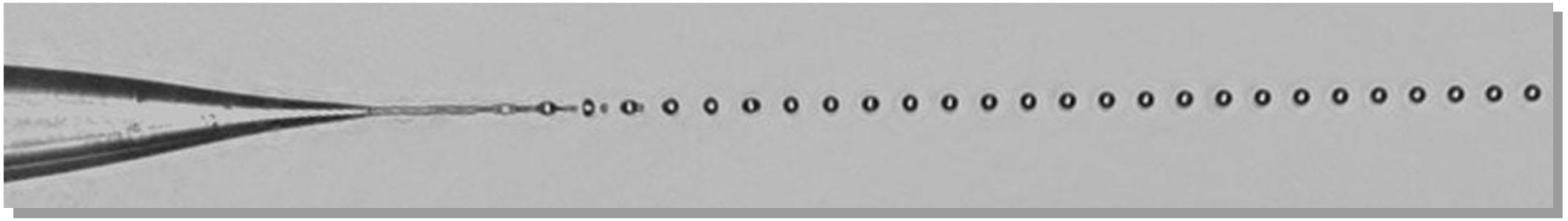
Electrospray in air

Shows breakup, water shedding.

8 micron Nozzle produces 1 micron droplets

Droplets freeze at 10^6 °/sec in vacuum,

Fast enough for vitreous ice.



Rayleigh jet in air. Can be synchronized with FEL.

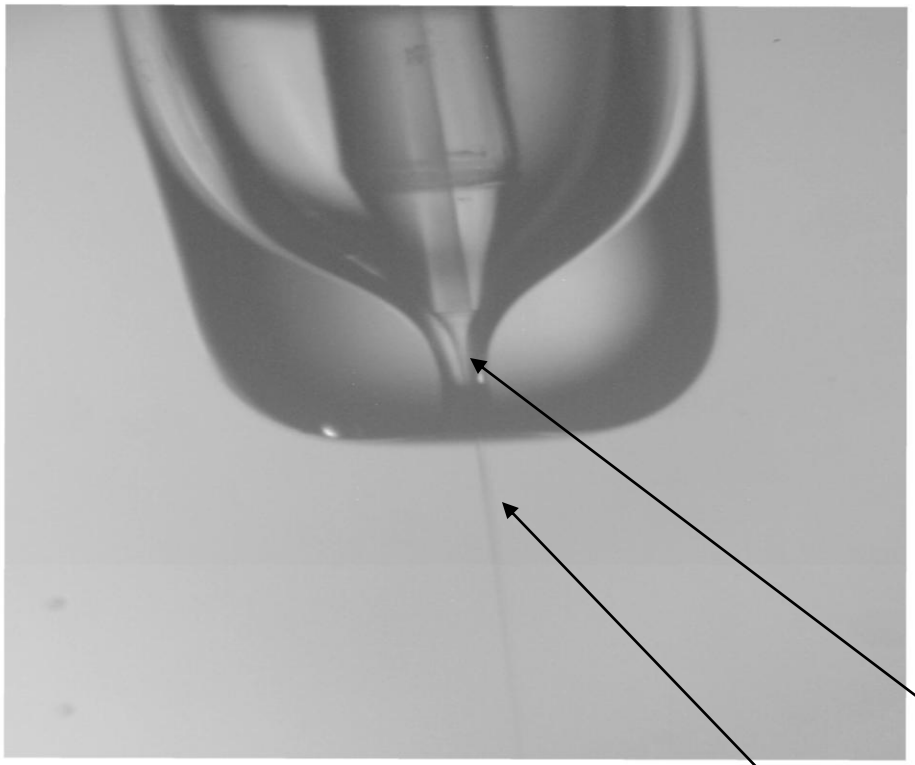
4 micron nozzle produces 8 micron droplets

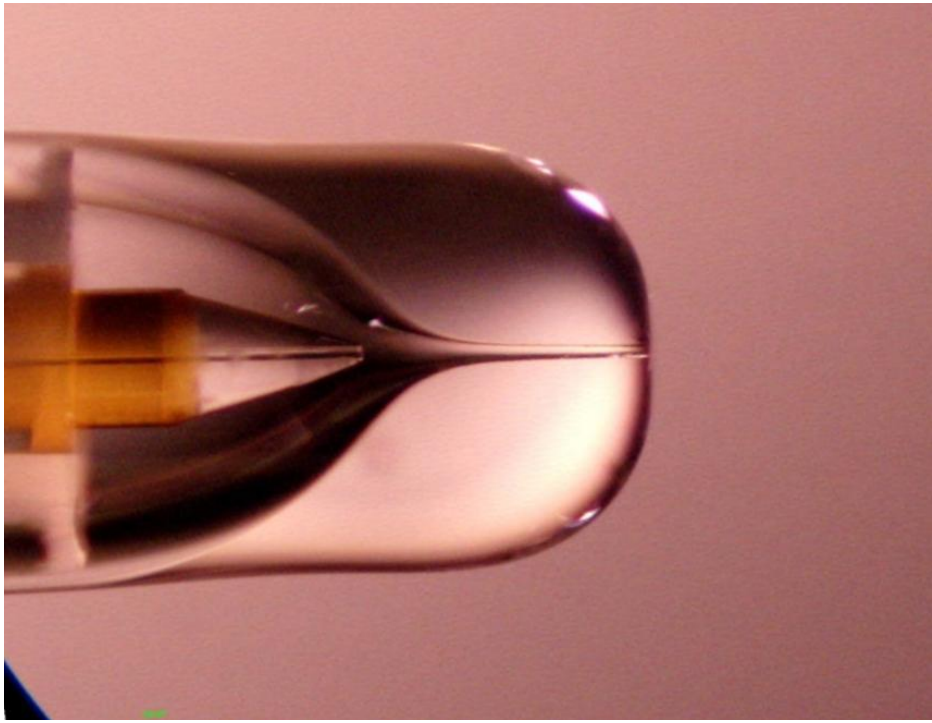
Piezo drive produces monodispersed drops, equally spaced, phase-locked to the driving waveform

The piezo-driven nozzle is vibrating at 1.2 MHz and the image obtained with 100 nsec stroboscopic flashes (LED) synched to the piezo. Droplet velocity 50 meters per sec. (Mag: 200)

U. Weierstall

Gas focussing prevents clogging - get submicron droplets from a bigger nozzle





Liquid cone enhances flow alignment

Av = const
Gas accelerates liquid
Making cone as shown.
Higher pressure for smaller droplets

~1 Mhz single-file micron-sized droplet beam. Big nozzle makes Small droplets

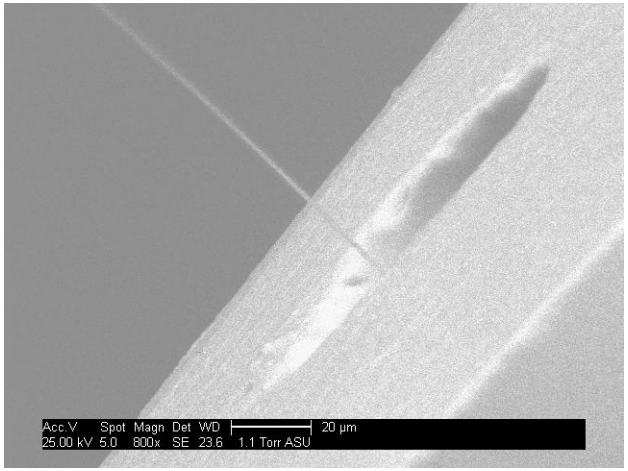
Operating Aerojet. Note liquid cone. Can be synchronized with FEL

Dan DePonte, Mark Hunter

We can image jet in SEM to see sub-micron drops

When droplets are too small for optical viewing.

Dan DePonte



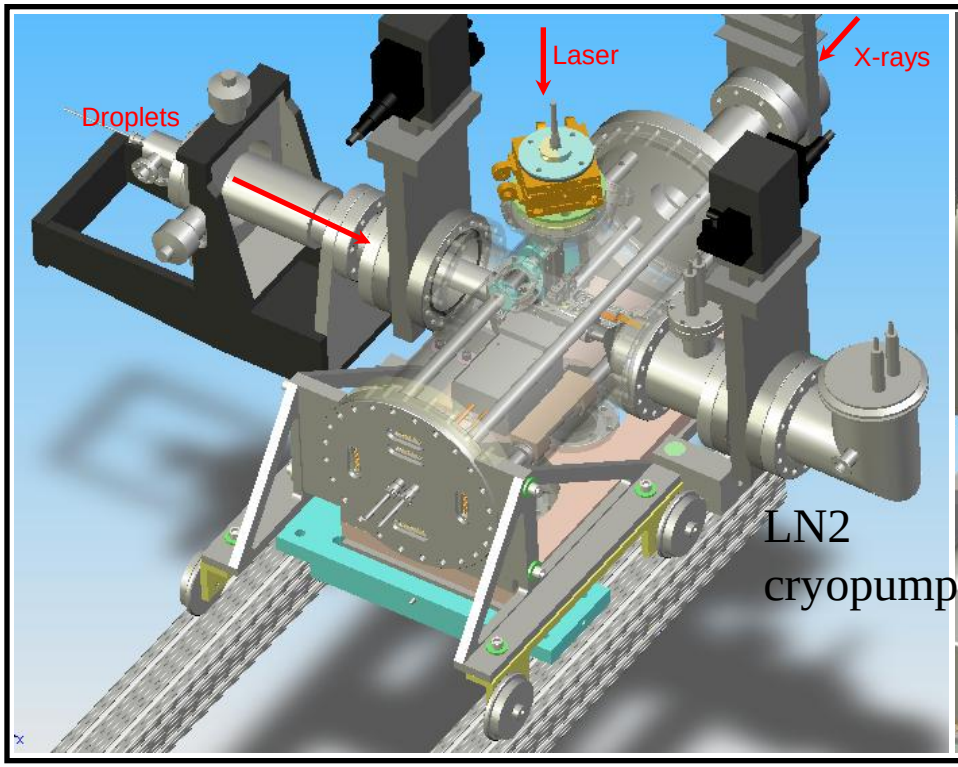
A submicron droplet beam (800nm) has been achieved

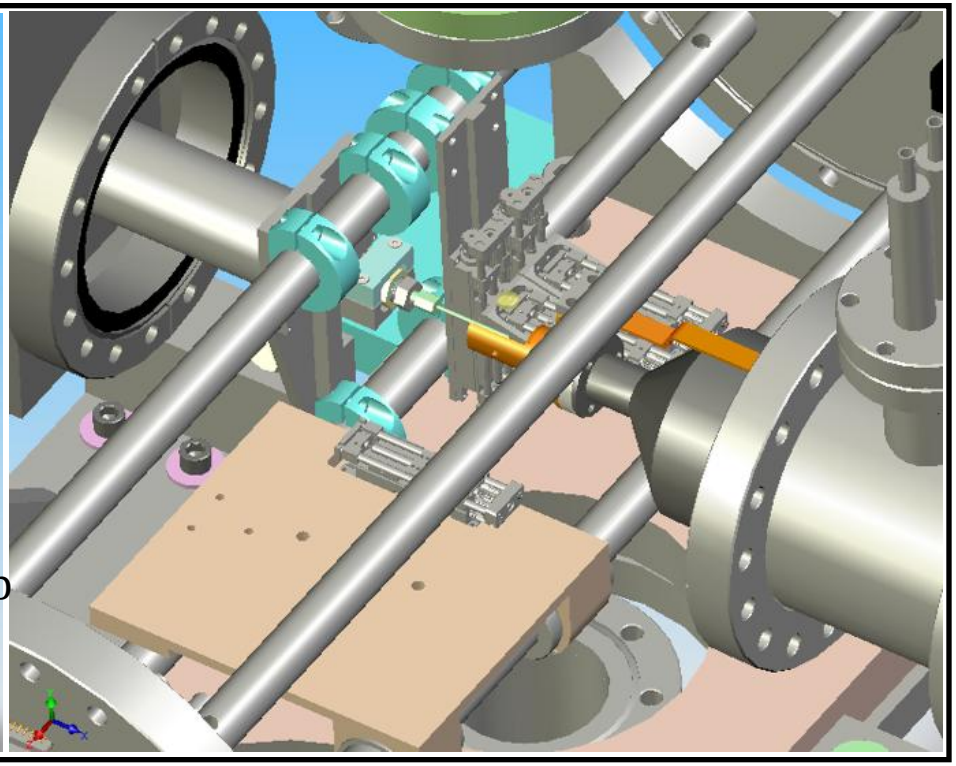
Uses of aerojet.

1. On-demand version can be synchronised with LCLS. Electrospray cannot.
2. Less damage than SAX cell, esp. for next gen synchrotrons. No window
3. Droplet jacket may act as tamper.
4. By reducing filter size, we can reduce crystallites to single molecule.
5. Use all liquour in protein xtal growth cells to improve yield (Von Dreele)
6. Windowless SAXS. Aerojet beam is thin enough for soft X-rays.

Tests of Diffraction Camera for S.C. and fs XRD at ALS

- Retractable nozzle and cold trap
- Adjustable sample/detector distance (3.5-5.5 inches)
- Computer controlled optics/laser positioning
- Maximum spatial frequency corresponds to 2 nm pixel size (after upgrade to 1.5 keV)
- Fully enclosed laser system





First experiments with soft X-rays at 2 nm wavelength at LBL Berkeley

D. Shapiro

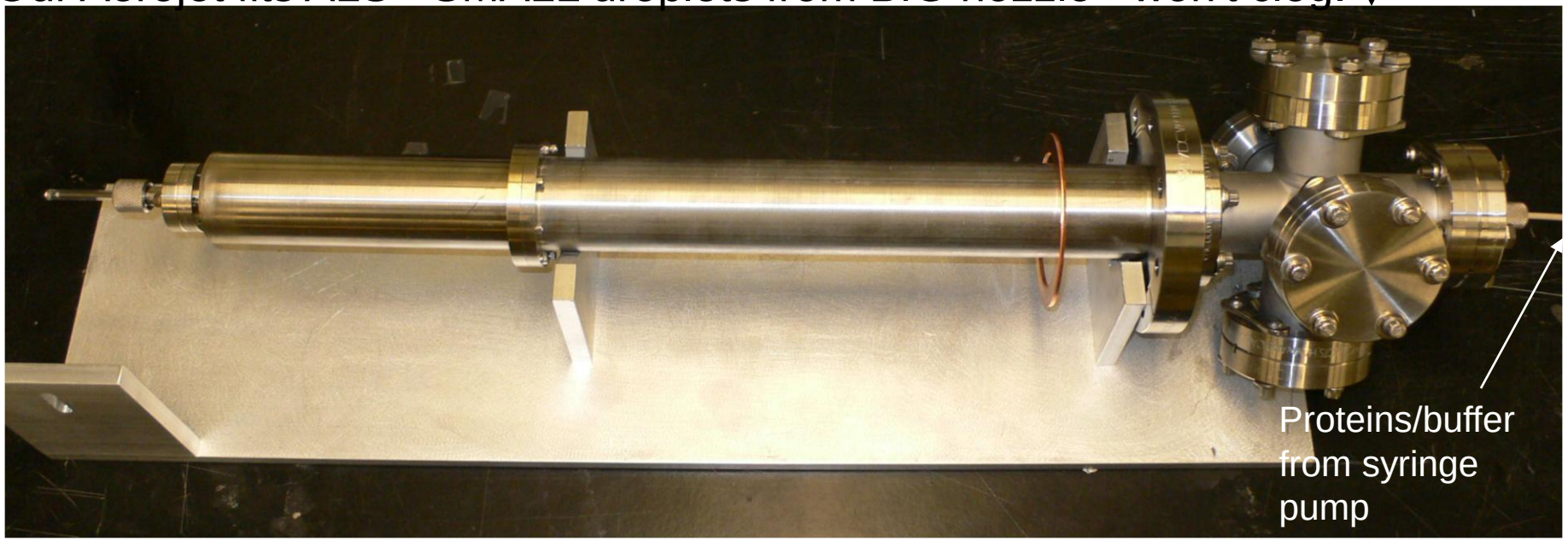
Aerojet test with CW X-rays.

1. 50nm gold balls in droplet beam

2. Photosystem I membrane protein 200nm crystallites.

3. Genome-free MS2 virus capsids.

Our Aerojet fits ALS - SMALL droplets from BIG nozzle - won't clog.



We have obtained diffraction patterns from 5nm gold balls doped into a single-file droplet beam.

50nm gold balls at 5 nm resolution in aerojet (2.3nm=λ, 530 eV) 1 ball/cubic micron of water.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

DePonte, Shapiro, Hunter

Horizontal line is from jet (rotated by 90 degrees).

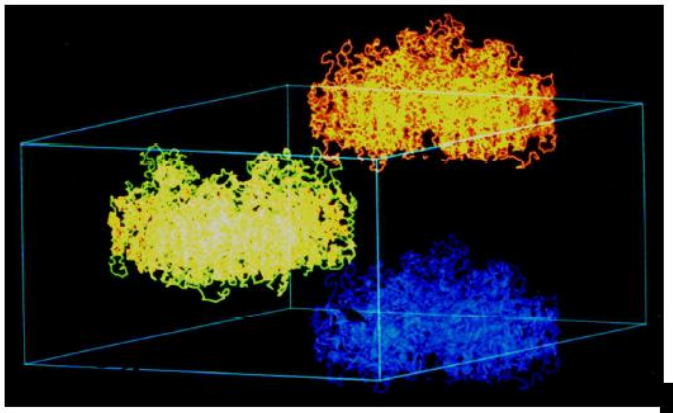
The aerojet was tested using protein crystallites of Photosystem 1

Photosystem 1 Protein was extracted from Thermosynechococcus Elongatus bacteria by HPLC, and xtallized in detergent micelles. Half-micron filter used.
(P. Fromme and M. Hunter, ASU)

PDB 1JB0

Crystal Packing

~ 1 MegaDalton
72,000 atoms

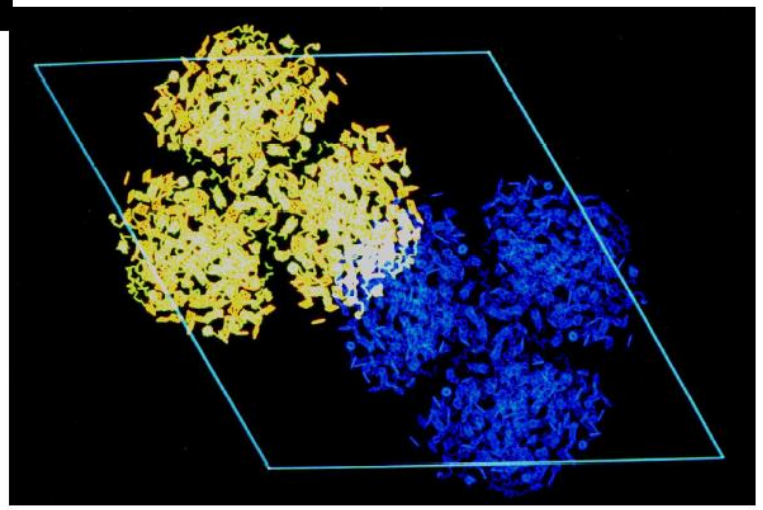


Space group $P6_3$

solvent content 78 %

a=b= 288 Å

c= 167 Å



Two trimers per unit cell.

P. Fromme, M. Hunter.

Many nanocrystals per droplet: A primer on powder diffraction

1. Sequence of ring radii gives Bravais, reciprocal lattice

2. With perfect instrumental angular resolution, *every ring is a different structure factor* for most proteins ($P2_12_12_1$,<=Orthorhombic). Then powder data would be as good as single xtal !

3. Four kinds of ring-broadening cause overlap:
   i) Energy spread in beam etc. causes beam divergence thro bragg law. Density of rings increases as ~ V Θ^2/λ^3. Energy plays like CL.
   ii) Particle broadening $\Delta\Theta/2\Theta$ = 1/(#cells per side) = $\Delta E/E$. Indep of λ.
   iii) Beam divergence.
   iv) Indexing accidents - none if symmetry orthorhombic or lower.

Scattered intensity varies as λ^4.

Conclude: Use lowest energy consistent with resolution needed. Good mono !

5. No azimuthal smearing as in gas, fiber, SAX. Solves orientation problem !

6. Heroic recent feats by Von Dreele, Irene Margiolaki (atomic res, mol rep).

Testing the jet with crystallites suggested a method for obtaining damage-free Powder Protein diffraction data from invisible crystallites in solution.

The efficiency from gene to protein to solved structure is currently 6%.

Increase efficiency by using invisible crystals normally wasted.

Efficiency could be greatly improved if ALL the mother-liquor in growth cells could be used, delivered eg by syringe pump into our aerojet. Prot Struct Init. records unsucessful m.p. crystallizations - check these solns.

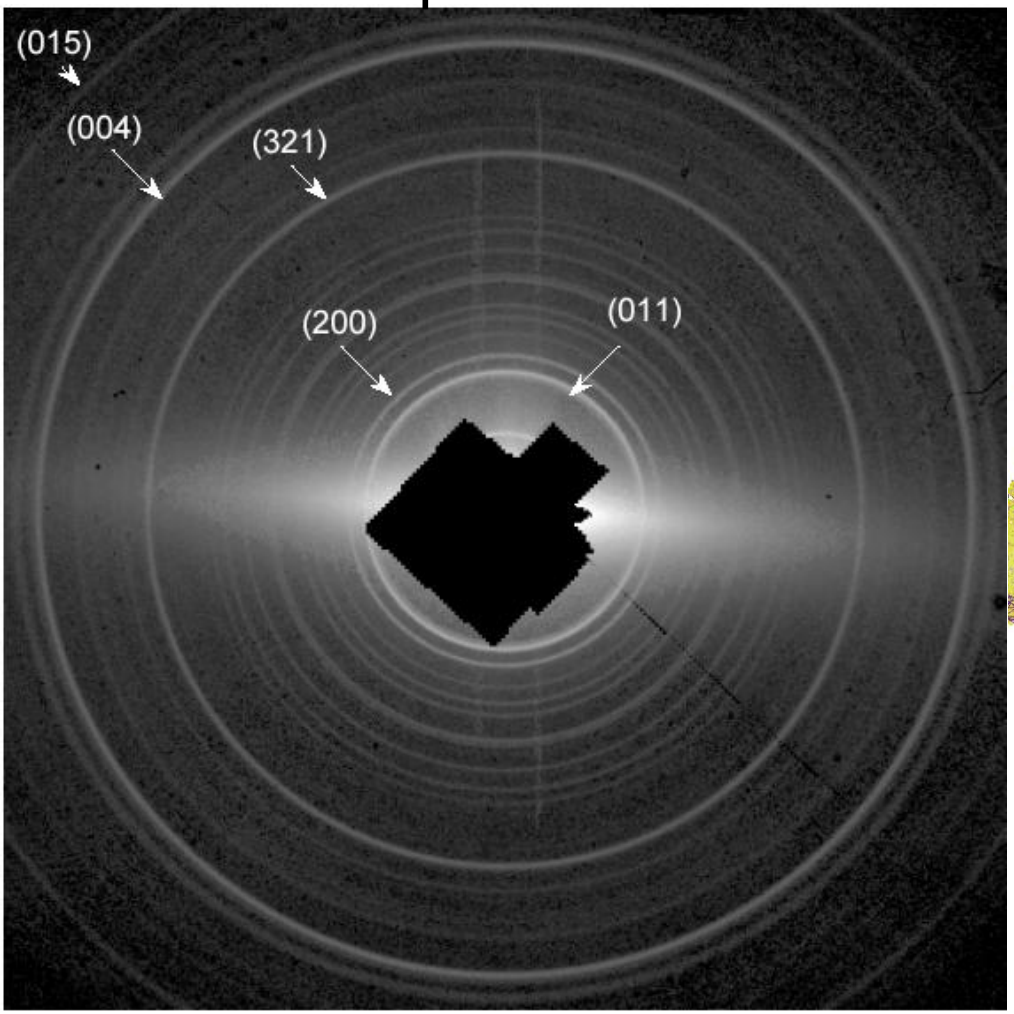
The use of the jet provides

1. Thin stream. $I \sim \lambda^4$, t_{ab}=16 mic, so use lowest energy, thin.
2. Temp variation along jet from RT to freezing to help resolve fine structure
3. No windows, as in SAX cell.
4. Flow alignment. We have patterns showing flow alignment from PS1 in liquid column, not drops.
5. Opportunity to screen invisible crystallites.
6. No damage.

Powder-Protein data from PS1 in aerojet at 1.5 kV (8 Angstoms)

Resolution in corner 2.8nm. d(015)=3.3nm. No. of unique reflections 244.

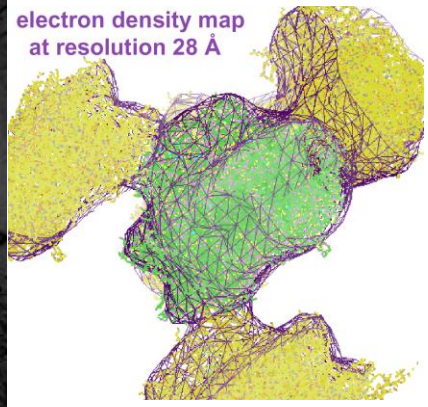
Experiment

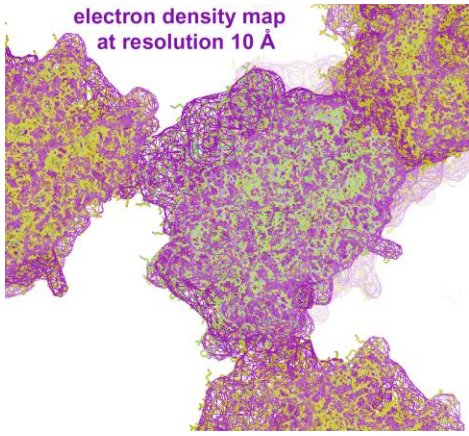


Crystallite size < 17 x 17 x 30 unit cells. Jet diam 10 microns. X-ray beam diam 50 microns

First powder protein data from membrane protein ?

Smallest xtals ever ?
17 unit cells on a side.



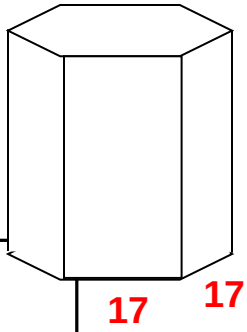


Our plan: Shrink nanoxtals to single mol size in LCLS by filtering.

Shapiro, DePonte, Hunter 08

Powder pattern from PS1 (1JB0) at 1630 eV using 500 nm filter. Exposure time 3.3 mins, which used about 200 microliters of PS1 solution (5mg/ml ?). Scattering angle in the corner is 15.8 degrees.

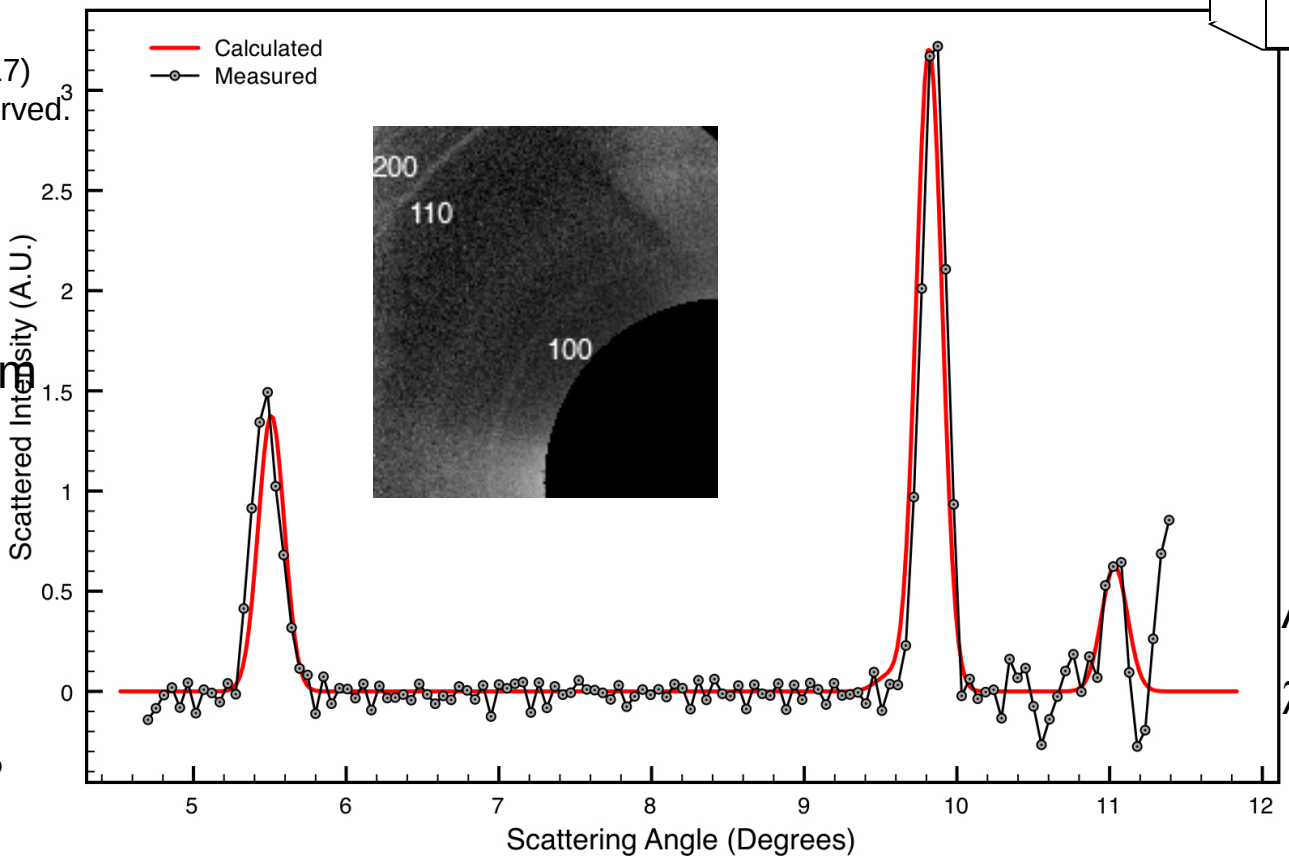
Intensities fits well for three lowest-order rings….



Size broadening
lambda/W ~ 23.6/(280*17)
= 0.28 degrees as observed.
Instrument is not limiting

Photosystem 1
72,600 atoms
$P6_3$, a=b=28.8nm
c=16.7nm

These xtallites
17x17x30 cells
(2 trimers/cell)
17,340 mols
-smallest ever ?



ALS

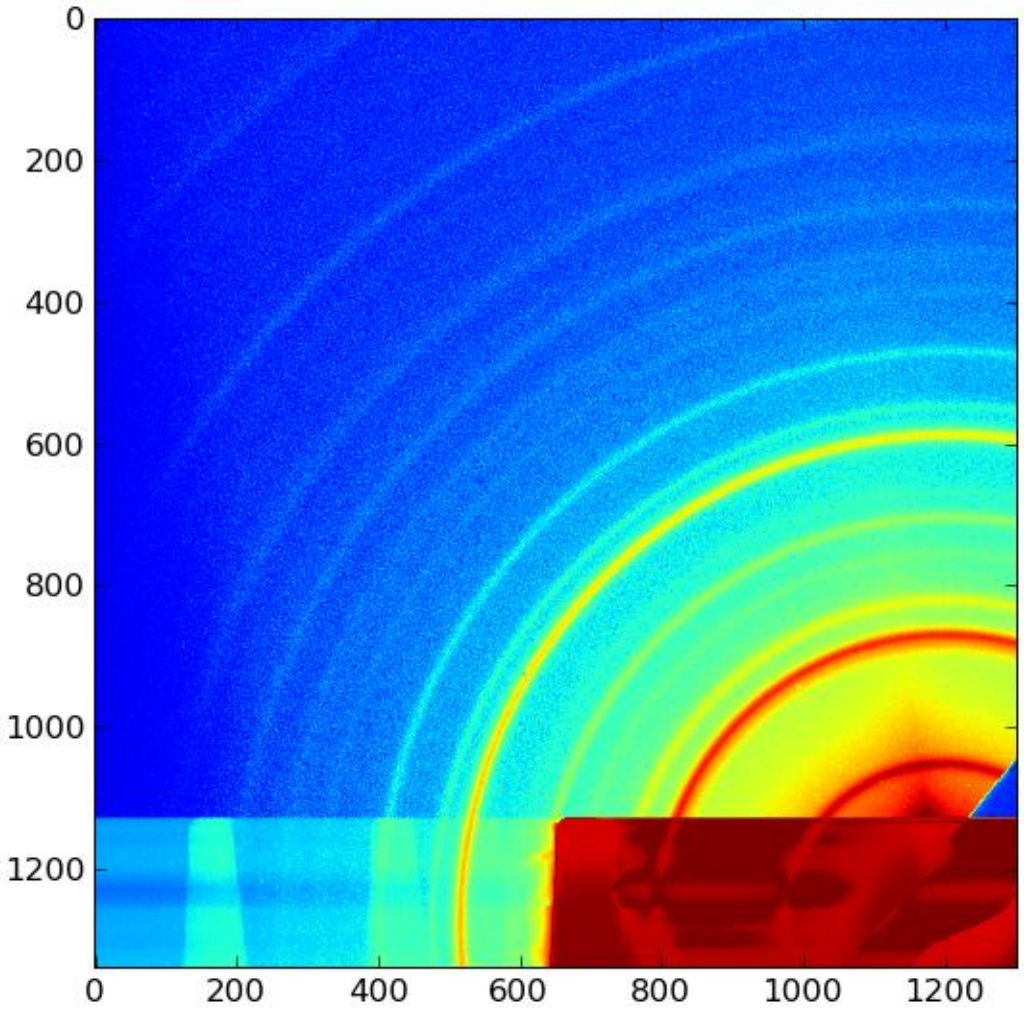
λ=2.34nm

Powder pattern from photosystem-1 nano-crystals with sizes less than 500 nm. Solid red curve - calculated powder pattern. Inset: experimental data with background subtracted.

We use 5 microliters of PS1 solution for 0.5 minute exposure. COSMIC

Shapiro et al J Synch Res 2008.

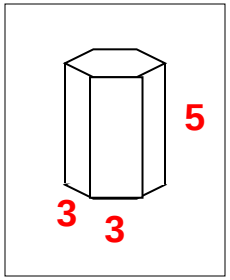
Powder membrane protein diffraction from **200nm** crystals of PS1 with soft Xrays



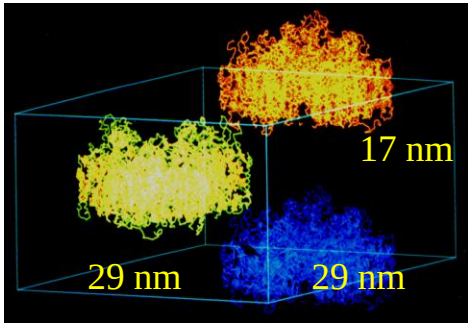
Why use soft X-rays ?
*Intensity goes as λ^4
*Resolution not limited by wavelength.

200 nm Xtals of PS1 at 520 eV (2.4nm wavelength).

XRD at 520 eV from **100nm** PS1 crystallites in aerojet
using 0.1 micron filter after HPLC syringe pump



QuickTime™ and a
decompressor
are needed to see this picture.

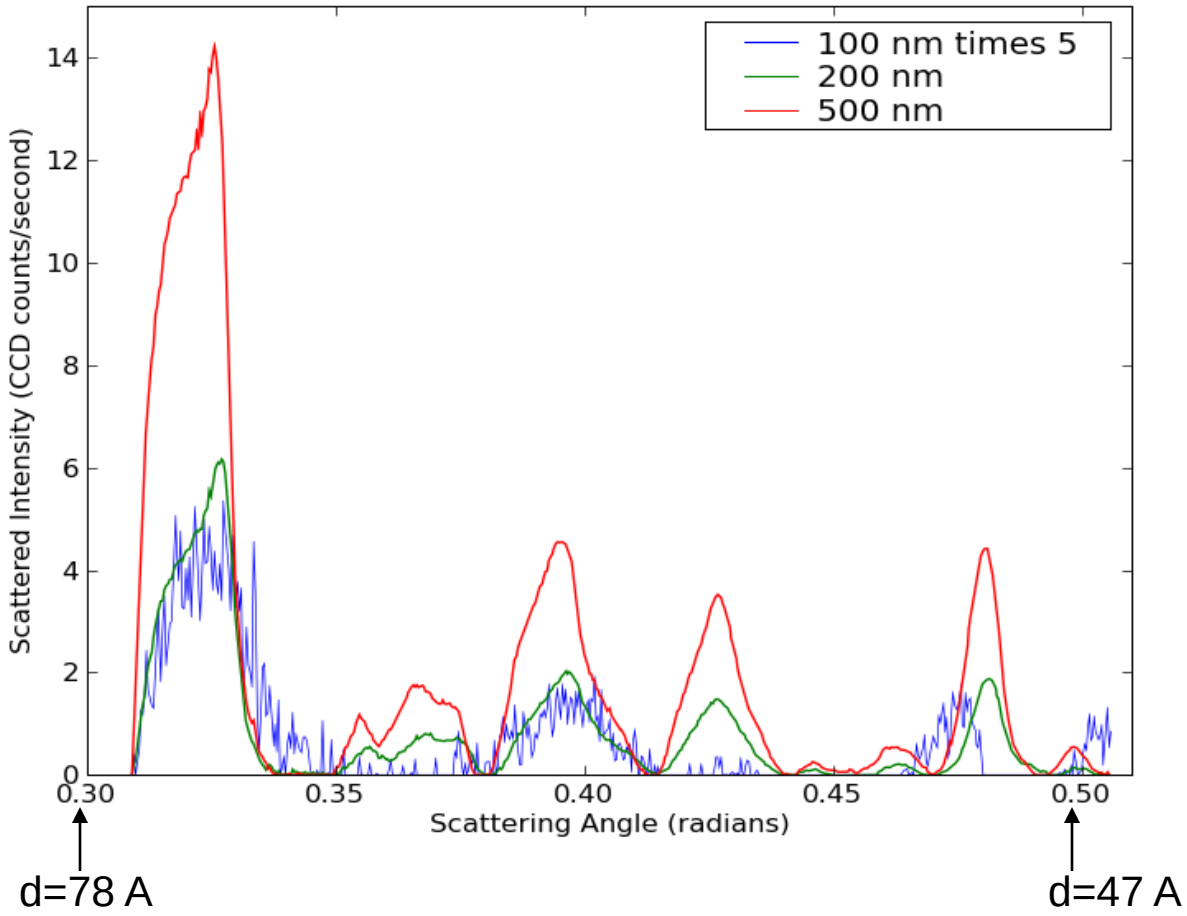


- 3 x 3 x 3 x 5 = 135 unit cells
- 270 PSI trimer molecules

100nm PS1 needle-shaped xtals in aerojet. Streak is diffuse from column of liquid.

Each xtal is about 135 unit cells, or 270 PS1 Trimers.

PS1 data summary - highest order rings.



CL=50mm, Energy 530 eV, 2.339nm wavelength

How to phase this data ?

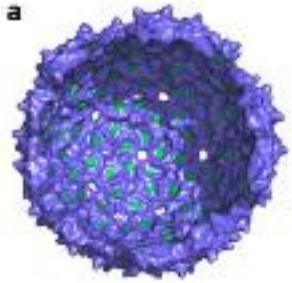
Direct methods require atomic resolution.
Iterative phasing requires oversampling.
(Sayre 51, Gerchberg-Saxton 73, Fienup 83, Bi-annual confs)

Reitvelt requires atomic res model for least squares.
Le Bail iterates Reitvelt intensities to refine |Fhkl|.
Pawley refines peak areas to get |Fk| for eg Shelex.
GSAS incorporates stereo-chemical restraints.
Wu, Spence, O'Keeffe Powder Flipping needs atomic resolution
(separates different $|F_{hkl}|$ on same ring Nature Mtls. 5, 647 (2006).
MAD needs Freidel pairs to be separated.
Multiple Isomorphous Replacement works for low resolution.

Marchesini's Compressive Sensing works for powder.

P $2_1 2_1 2_1$ is most common protein spacegroup. No overlaps due to indexing degeneracy

Aerojet CW XRD from genome-free MS2 viral capsids (24nm)



Aims

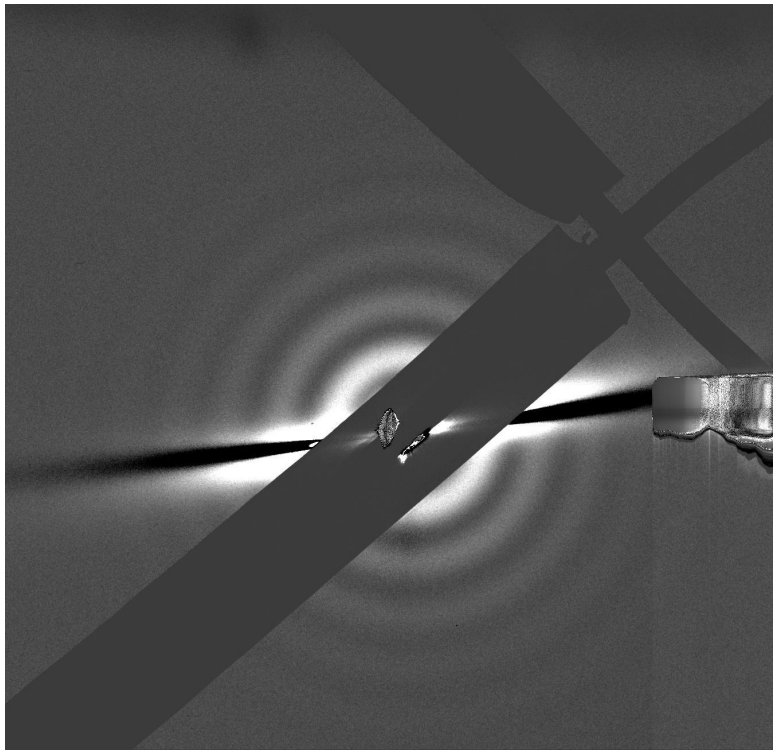
*Test aerojet using CW X-rays , find conc needed, evaluate background from buffer.

*Same energy as LCLS (2 kV) so evaluate resolution.

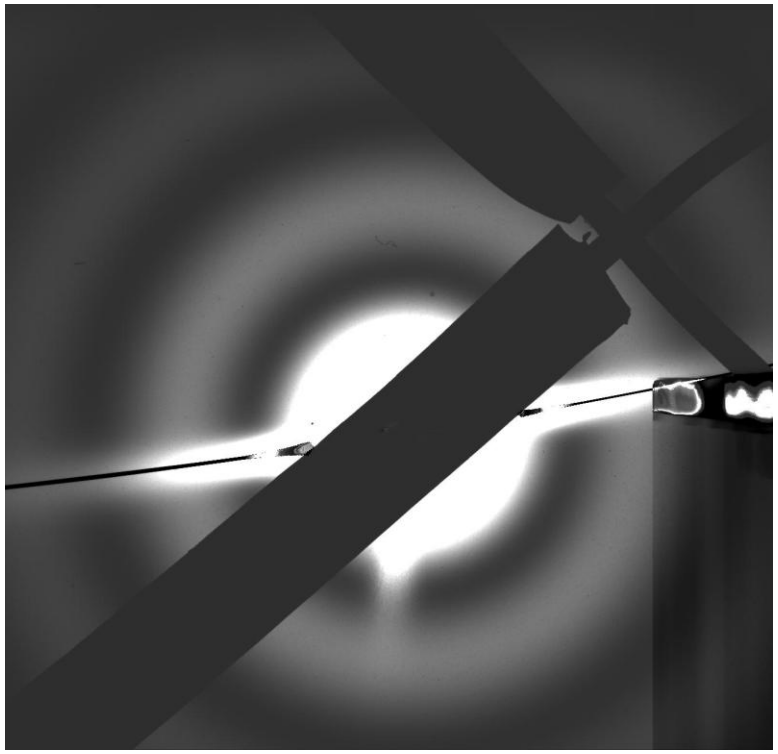
*Compare diam of capsid with value from Ion Mobility analysis, to compare Electrospray vs Aeroject (charging, dry ?)

*Scale ALS pattern to LCLS intensity - any LCLS counts at high angles ? cf N^2 vs N scattering.

Shapiro, Spence, M. Francis. Praveena Garimella et al ASU,LBL,UCB



MS2 1500eV 24nm diam

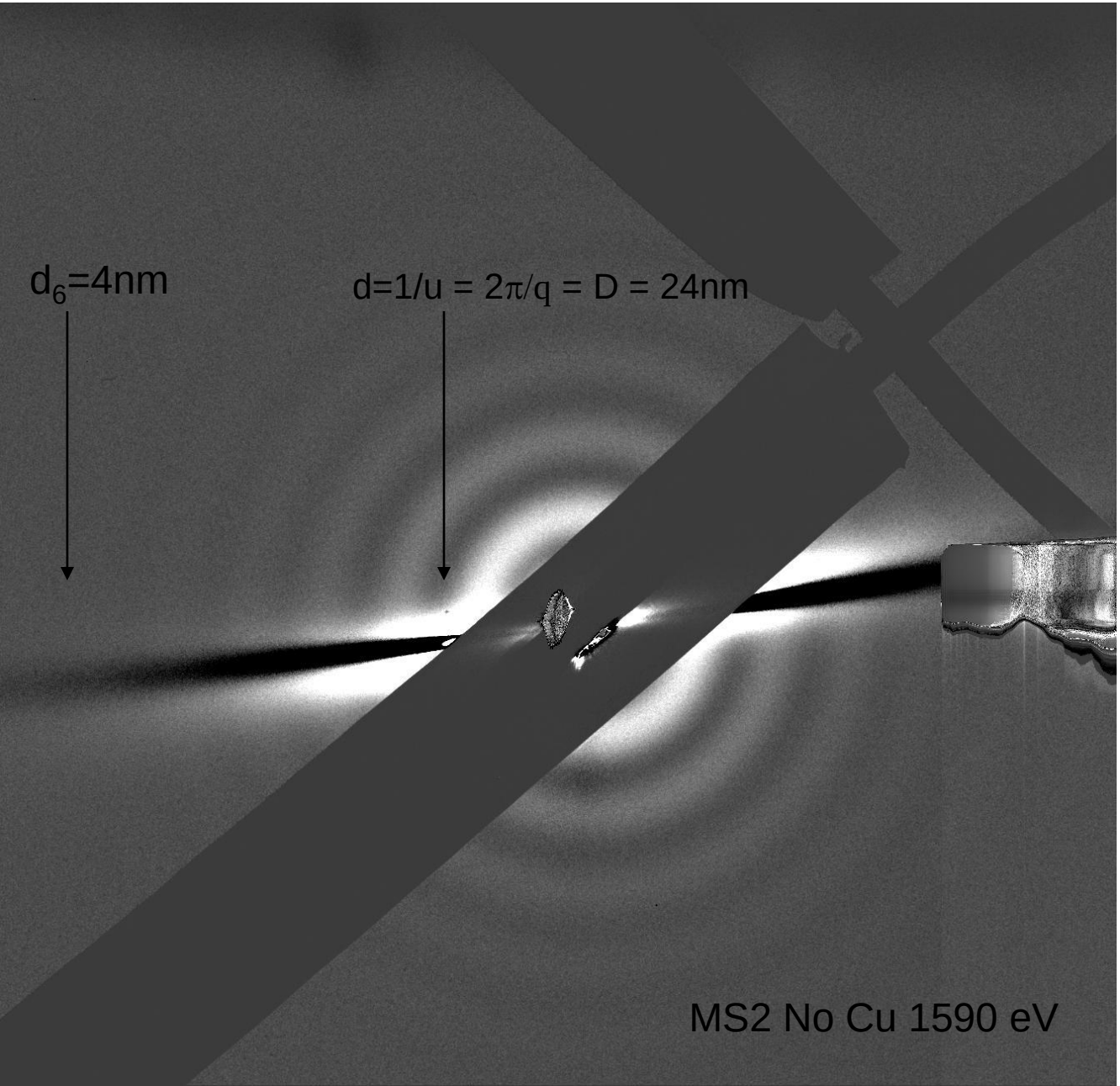


MS2 500 eV

QuickTime™ and a
decompressor
are needed to see this picture.

Genome-free MS2 in aerojet. No Cu, 1500 eV Log scale. Mat Francis

MS2 Capsids in Aerojet. 1.5 kV



FT of a thin spherical shell is a Sinc function

Ion Mobility analysis by electrospray gives diam of genome-free capsid as 23.6 nm*.

Difference may reflect different enviromt of electrospray (charged ? dry ?) and aerojet (lots of water)

SAXS gives 26 $\pm$ 0.04 nm ** (bead model).

Capsid with Cu gives 22.2nm

*Thomas, Benner et al 04
**Kuzmanivic et al 06

Intensity scaling for LCLS

Find counts per pixel at high angles if this pattern had been a single-shot pattern from LCLS.

Assume $N_s = I_o\ n\ \sigma$ holds at both ALS and LCLS (σ cross section, n particles (=1 for LCLS), I_o incident fluence.

Use 50 micron ALS beam, 3 micron LCLS.

I_o = 1E12 for LCLS (single pulse), I_o = 20E12 at ALS (20 sec exposure)

Since the average count per pixel at ALS was 1E6, it will be 17.4 at LCLS

The maximum LCLS count will be 1742 (with oversampling factor of 5)

Recent results from Flash

Pulsed Soft-Xray FEL at DESY, Hamburg

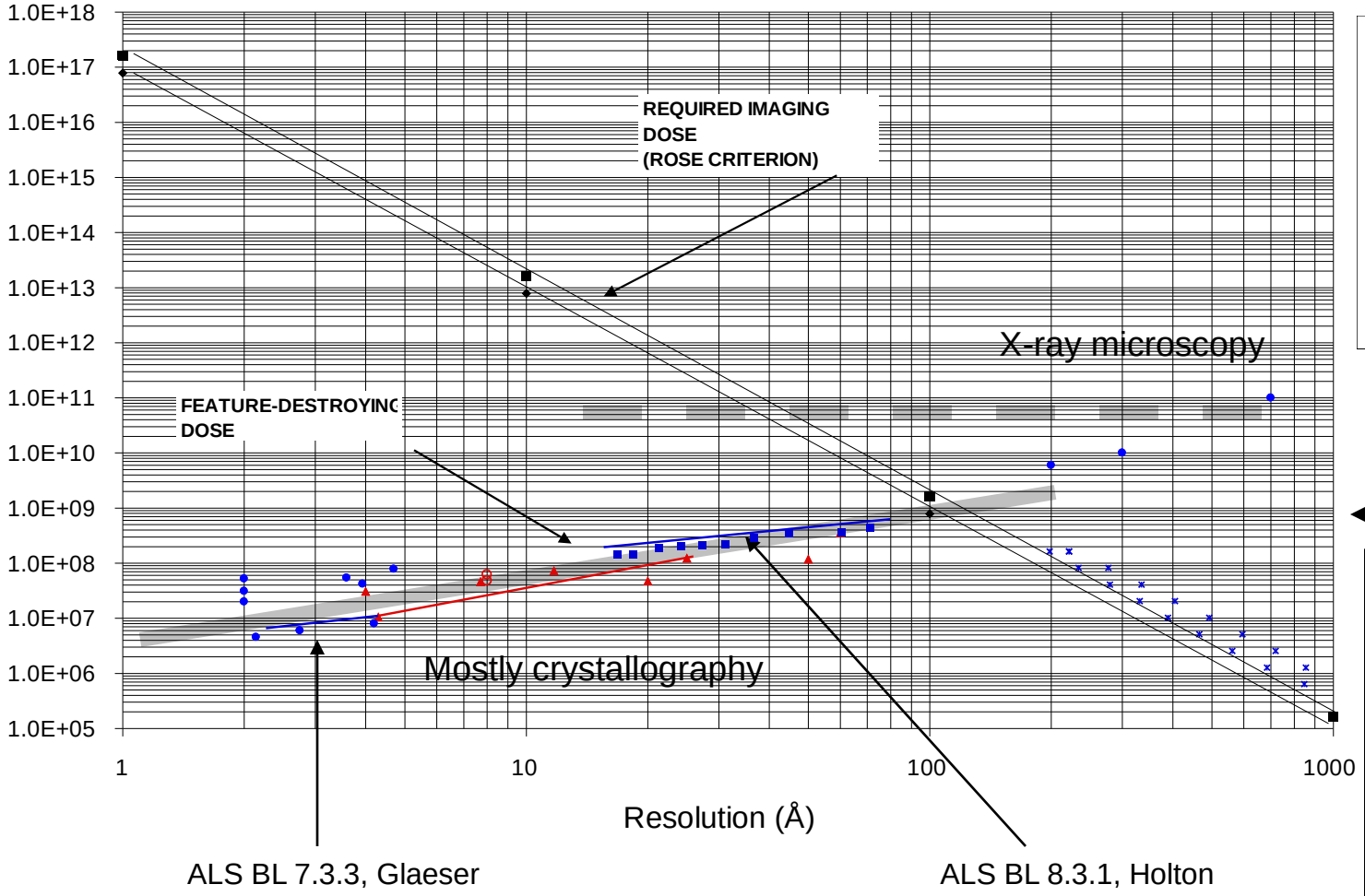
13.5 nm wavelength 10fs pulses
1E12 photons per pulse

H. Chapman et al.

DOSE vs RESOLUTION : 3D XRAY IMAGING OF FROZEN-HYDRATED SAMPLES

Conclude: Dose ~ Resolution^{-4} Coherent scattered flux ~ λ^4.

WE CAN NEVER DO BETTER THAN 10nm ! How to break this nexus ? Use time domain



Every bond broken above here

X-rays:
Glaeser et al 2000
Gonzales et al 1992
Sliz et al 2003
Burmeister 2000
Schneider 1998
Henderson 1990
Maser et al 2000

Electrons:
Glaeser and Taylor 1978
Plitzko et al 2002
Hayward and Glaeser 1979

Howells et al J. Elect Spec Rel Phenom O8

There is a continuum of damage processes, each with its own timescale.

Can we "outrun" these timescales with a sufficiently short exposure ?
Can we pack enough photons into this time to get a decent pattern ?
Expts show that **dose goes as resolution^{-4}** ! (Howells et al 08).
Atoms (nucleii) don't start to move till about 100 fs.

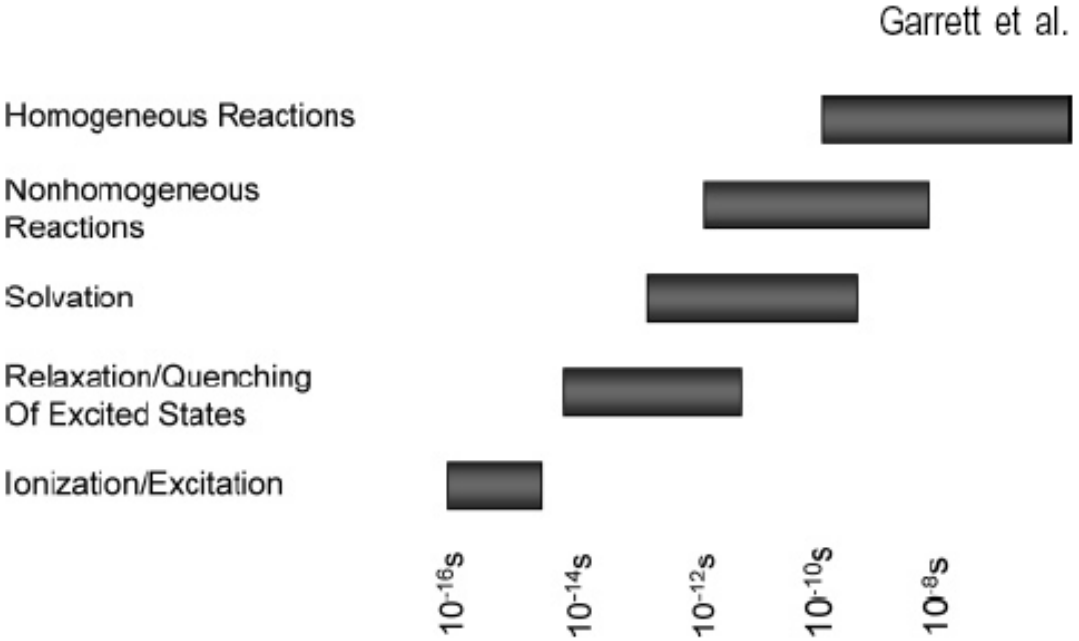
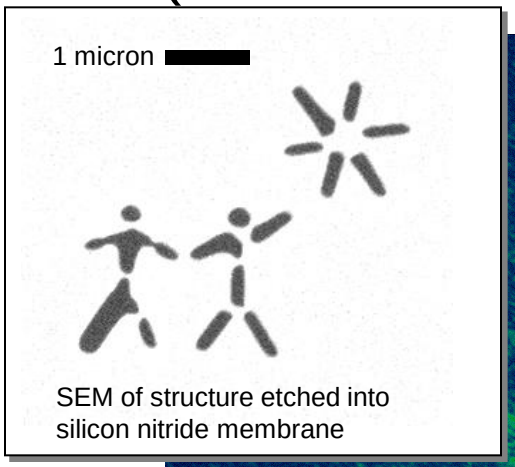


Figure 2. Approximate time scales of processes initiated by ionizing radiation.

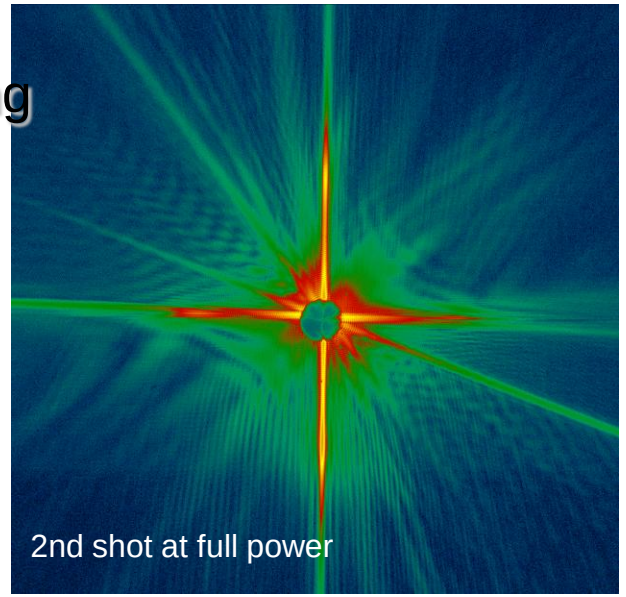
<u>If an X-ray pulse stops before a particular time, that process (eg hydrogen bubbles) cannot contribute to the diffraction pattern.</u>

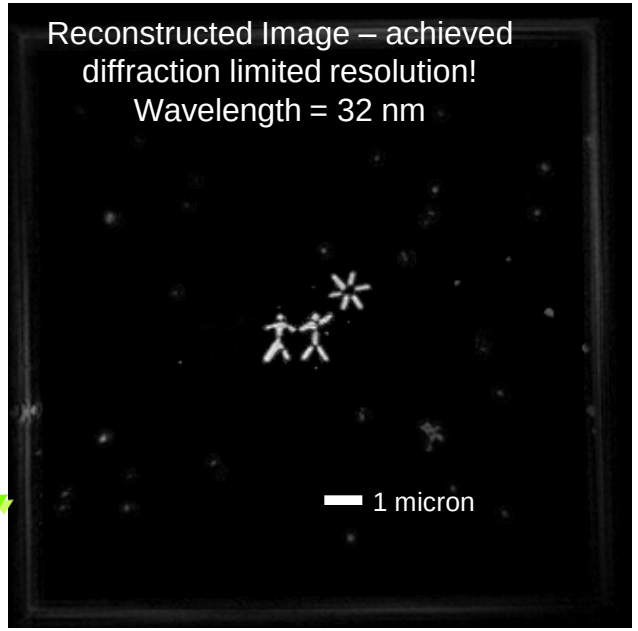
X-rays are diffracted by the electronic charge distribution, which redistribute on the time-scale of Auger recombination (a few fs). Nuclei (100fs) don't diffract X-rays.

Image reconstructed from a FEL diffraction pattern . (DESY VUV-FEL) 25+/- 5 Femtosecond pulses



SEM of structure etched into silicon nitride membrane





H. Chapman, A. Barty, S. Marchesini, J. Hajdu, et al Nature Physics 2, 839 (2006)
10^12 photons incident per 25 fs pulse.

Edge of membrane support also reconstructed

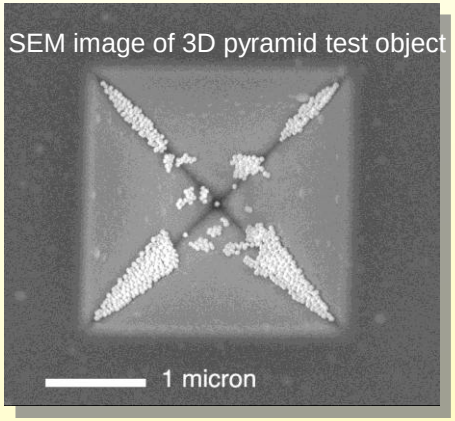
How is the phasing done in 3D for this CW "lensless imaging" ?

Ab-initio 3D image reconstruction of non-periodic object at 10nm resolution

X-ray diffraction data collected at the Advanced Light Source, LBNL, at a wavelength of 1.6 nm, from a sample of 50-nm gold spheres arranged on a pyramid. **Resolution 10nm. 3D**.

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

Coherent X-ray diffraction data, rotating the sample -70 to +70 degrees



Diffraction patterns from non-crystals are continuous and contain more information than can be obtained from crystals (limited to Bragg spots)

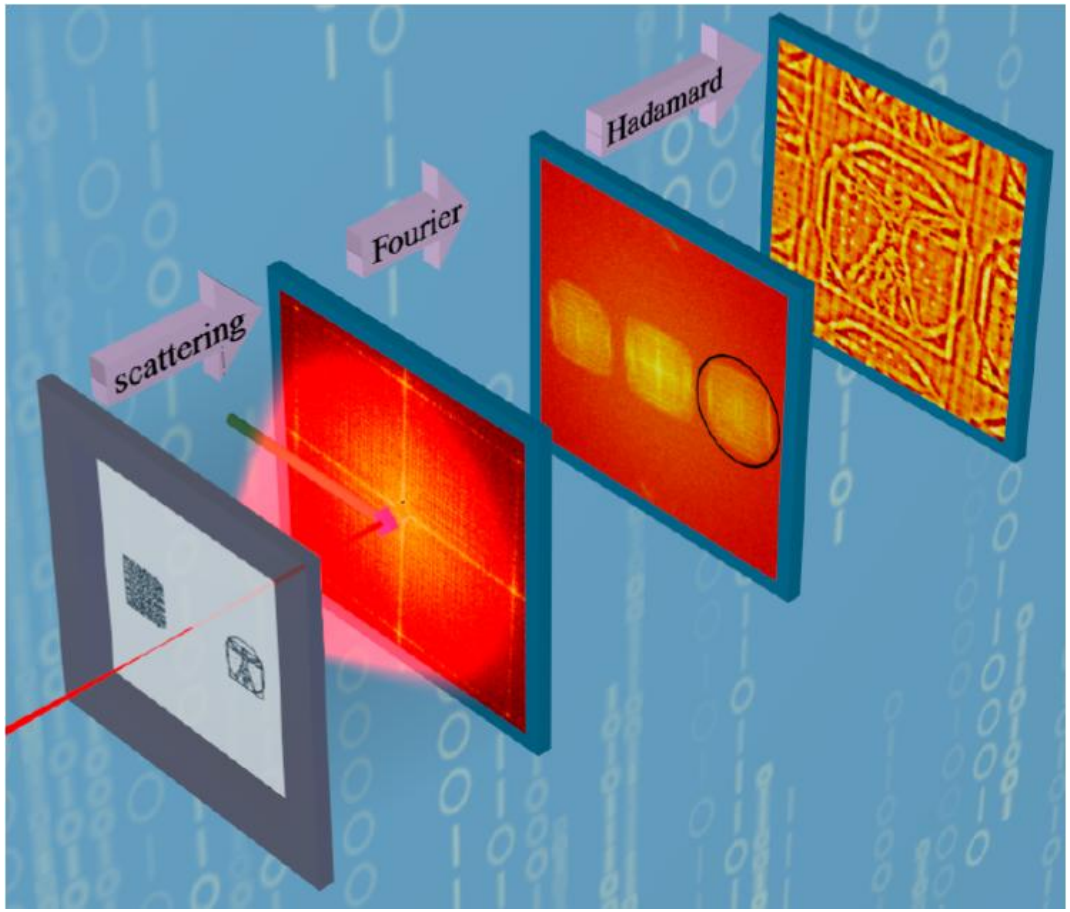
This allows complete image reconstruction without the need for any prior knowledge, using our “shrinkwrap” algorithm to reconstruct the image.

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

Chapman, Shapiro, Barty, Marchesini, Spence et al J. Opt. Soc Am 23, 1179 (2006)

Cf Paul Midgely TEM, thinner

Fourier-Transform holography with femtosecond X-rays.



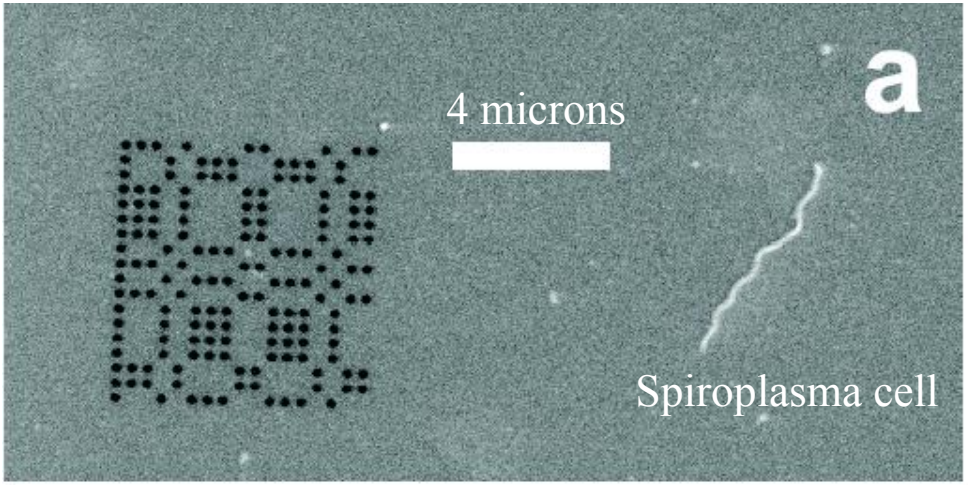
With a point reference object, the FT of a satellite distribution gives direct, *non-iterative* inversion.
We use a Hadamard transform to deconvolute the effect of an extended (URA) reference.
URA provides stronger reference than point - reduced dose, exposure. URA has flat power spectrum.
Improve resolution further by iterative phase extension.

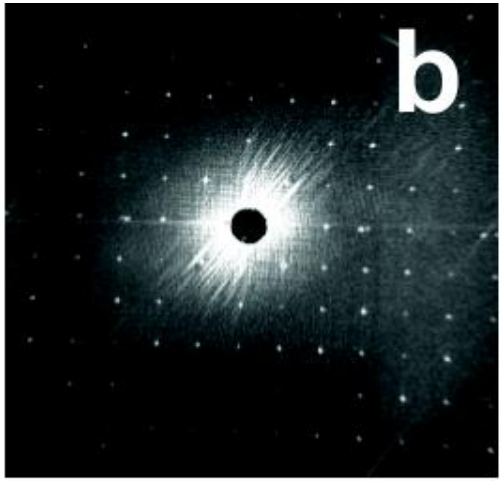
Marchesini et al Nature Photonics 08 Work at ALS and Flash (DESY).

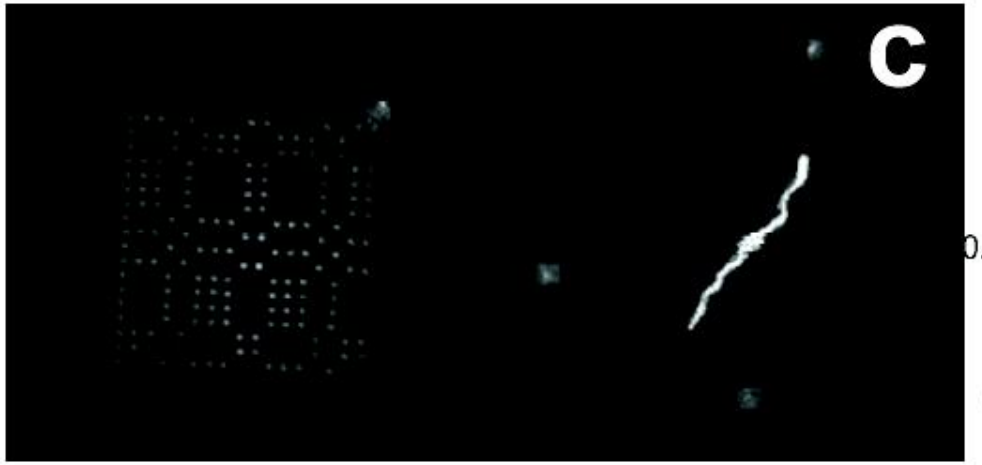
Diffract-and-destroy at Flash (DESY). Single Shot

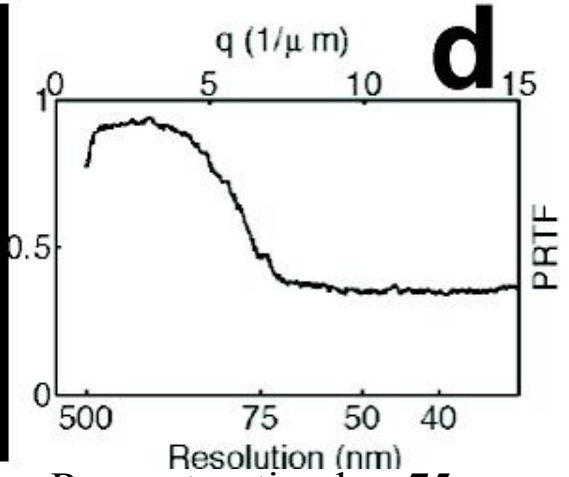
XRD 10^{12} photons in one 15fs pulse at 13.5nm with 20 micron spot λ=13.5nm

URA has 150 nm resolution









Reconstruction with iterative phase extension.

Reconstruction has 75 nm resolution
Better than URA !!.

Flux efficiency suggests use for cavity-HHG "tabletop" X-ray lasers.

Marchesini et al Nature Photonics 2008

Summary

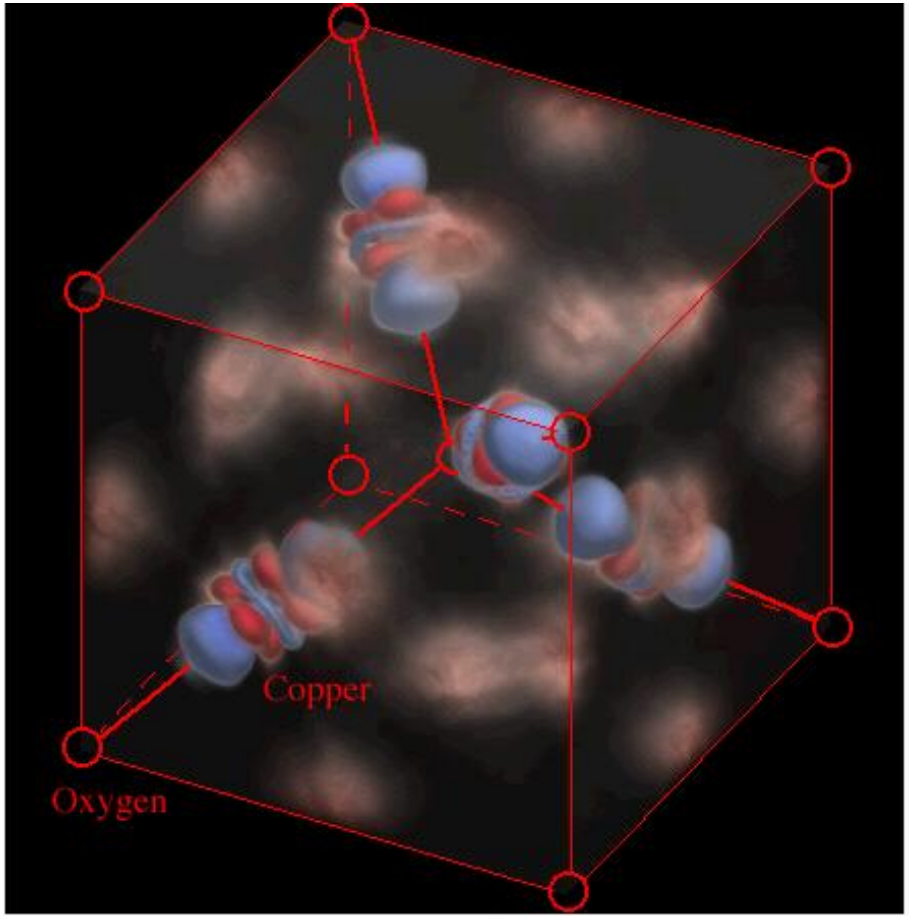
1. Diffraction from laser-aligned mols has been achieved (*not hydrated*).
2. 3D diffractive (lensless) imaging achieved (pyramid of gold balls) at 10nm res.
3. New Aerojet sources designed, tested which don't clog, makes uncharged, micron droplets and can be synchronized with LCLS. Tested at ALS on PS1, Capsids.
4. Charge-flipping iterative phasing algorithm adapted for Powder (Wu et al Nature Mat).
5. Correlations in few-particle "SAXS-PLUS" detected. Inversion works for gold rods.
6. Inversion problem of multiple scattering solved for 2D object (Spence, Acta A 09).
7 "Diffract-and-destroy" principle established at Flash. Single-shot image obtained from a cell.
8 The aerojet works in a TEM, where the e- cross section is much greater than for XRD.

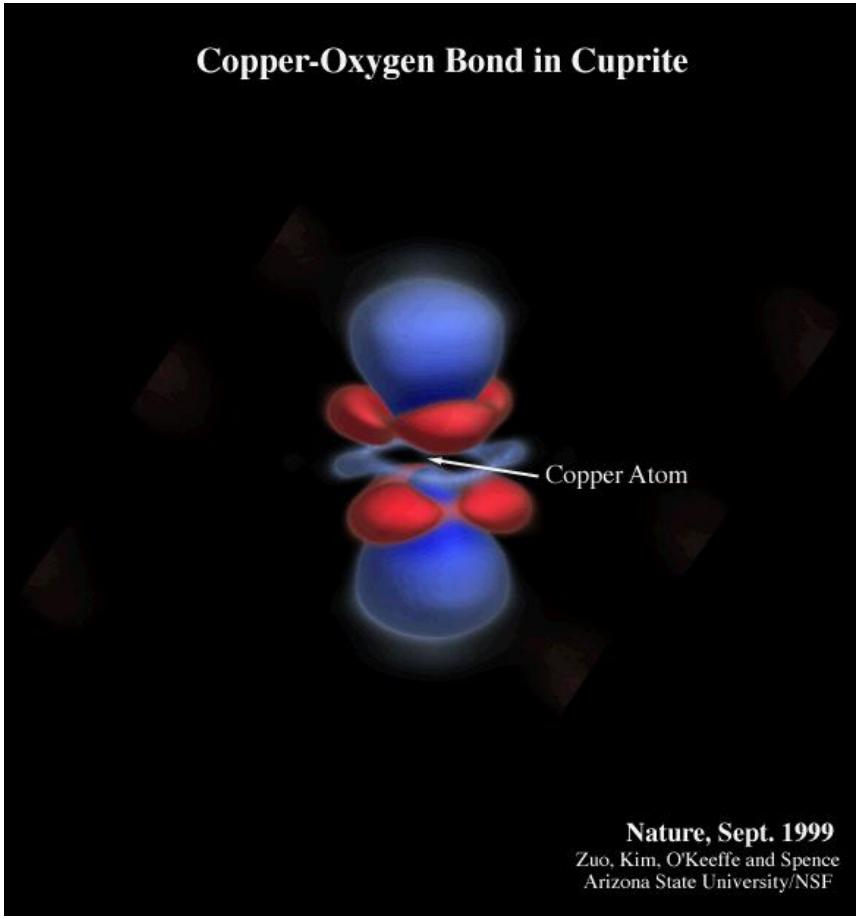
IMMEDIATE AIMS:

1. **Synchronized aerojet for LCLS. 2009 - Spot fading vs pulse duration**
2. **Femtosecond crystallography of protein nanocrystals. Readout each shot**
3. **Fluctuation "SAXS-PLUS" of protein nanoxtals in ice (NSLS2 ?).**
4. **Fs XRD of viruses, macromolecules ? (Single shot).**

Comment on microbeams: Microdiffraction in TEM uses 1nm beam (see Spence and Zuo "Electron Microdiffraction". Plenum 1992

“Seeing Bonds”Quantitative convergent beam electron diffraction in TEM





Surface of constant charge density difference in Cu_2O.

Total measured charge density minus calculated spherical ions on lattice sites.

If cuprite were purely ionic ($Cu^{+}O^{--}$) this picture would be blank.

Blue is less charge than MCDF ions (making "holes" in the shape of d_z^2 orbitals), red is more.

Coppers form FCC lattice. Image shows deviations from textbook ionic bonding, e.g dz^2 hole, covalent metal-metal bonding. 0.22 electrons of charge move from the hole into this bond.

Copper-copper bonds seen experimentally are not predicted by FLAPW/LDA theory.

Results are accurate enough to distinguish between many-e approxs (LDA, GGA).

*Zuo,Kim, O’Keefe, Spence, Nature, 401, p.49 (1999)

Cu+ $[Ar]3d^{10}\ 4s$ O^{--} $1s^2\ 2s^2\ 2p^6$ (2 unpaired)

C

Scanning Transmission Electron microscopy uses 1 Angstrom diam probe to spatially map inner-shell edges

QuickTime™ and a
decompressor
are needed to see this picture.

X-rays will give worse "resolution" but better quantification

Muller et al Science 319 p.1037 (2009)

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DePonte et al, "Aerojet". J. Phys D. 41, 195505. (2008) Aerojet. 2008.
DePonte et al "SEM imaging of liquid jets". Micron 40, 507-509 2009.
Shapiro et al J. Synch Rad. 15, 593 ('08) Powder rings from Aerojet droplet beam.
Spence J. (2008) Multiple scattering inversion. Acta A. 2008.
Schmidt et al (2008) A scheme for femtosecond tomography. PRL 101, 115507 2008.
Wang et al. (2009). Molecular orientation determination from the moments of inertia of their diffraction patterns. Acta A. Submitted.
Kirian et al (2009). Reconstruct image of one particle using scattering from many. Science. Submitted.

People.

B.Doak, U.Weierstall. P Fromme, K.Schmidt, D.Starodub, D.DePonte,G.Hembree,
J. Spence, P. Rez, (ASU)
M.Howells (LBNL), D. Shapiro (LBNL), A, Barty, H. Chapman (DESY)

Money.

NSF SGER, ARO, NSF IDBR > Nov 06**.** NSF **CBST.**

The End

Faraday (from photo ! 1850 ?)
With the xtal in which he observed rotation of polarization of light due to an external magnetic field

"Theory is useless in biology"

(there's no Occam's razor).

Physics is a problem in search of a solution
Biology is a solution in search of a problem

Crick..
Biology is the reduction of mechanisms to chemistry

"Science is nothing but the gradual reduction of prejudice and ignorance'
(Richard Rhodes Oct 05)